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Case report

Chronic pericarditis in a naturally *Leishmania (Leishmania) chagasi* infected dog

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Abstract

Visceral Leishmaniasis is an infection disease of chronic evolution caused by the protozoan *Leishmania* sp. The main clinical manifestations in dogs are anemia, progressive weight loss, lymphadenopathy, hepatomegaly, and cutaneous lesions. The heart involvement in visceral leishmaniasis has been rarely reported. The aim of this work was to describe the presence of amastigote forms of *Leishmania* sp. and associated lesions in the heart of naturally infected dog. For diagnosis analysis, serological tests for anti-*Leishmania* antibodies and fine-needle aspirative bone marrow cytology were used. The samples (right ventricle and ear skin) were histologically evaluated and processed for imunodetection of *Leishmania* sp. The most significant histological change was an intense, non-specific, chronic pericarditis associated with intracytoplasmatic amastigotes within macrophages. The tissue parasitism was confirmed through positive imunomarcation. This is a first report of pericardium compromisement of naturally *Leishmania* infected adult dog.

Key Words: Heart; *Leishmania* sp.; visceral leishmaniasis; dogs.

Introduction

The clinical presentation of visceral leishmaniasis or kala-azar in man is variable but usually includes fever, severe weight loss, lymphadenopathy and hepatosplenomegaly (2). In immunocompromised patients the clinical course of the disease is even less specific and the diagnosis is often made by means of incidental detection of the parasites at atypical sites such as the gastrointestinal tract, peripheral blood, lungs and cerebrospinal fluid (1). A case report of visceral leishmaniasis with heart compromisement leading to pericarditis was described in HIV-infected patient (5,7).

Canine visceral leishmaniasis is a highly prevalent infection caused by the protozoan *Leishmania (Leishmania) chagasi* in Brazil (4). The infection can cause severe systemic disease with progressive weight loss, anemia, generalized lymphadenopathy, hepatomegaly, dermatological changes and onychogryphosis. During infection, *Leishmania* promastigotes infect cells of the mononuclear phagocytic system in various host organs such as skin, liver, spleen, lymph nodes and kidney (3). However, the heart involvement has been rarely reported in *Leishmania* infected dogs. Myocardial degeneration and necrosis and myocarditis with parasite DNA were observed, predominantly in the right atrium of an adult dog (9). *L. chagasi* has been also detected in the heart of puppies from an infected pregnant bitch (8). But none of these cases reported the pericardium involvement associated with *Leishmania* sp. infection as described in HIV-infected patients (5,7).

Case Report

An adult female dog positive in serological tests for anti-*Leishmania* antibodies and fine-needle aspirative bone marrow cytology was referred to the Diagnostic Service of Fauna and Flora Institute for postmortem examination. The animal from Center for Zoonosis Control in Betim (State of Minas Gerais) was submitted to euthanasia as part of the official program for zoonosis control with a lethal dose of sodium thiopental [2.5% (1.0 ml/Kg)] and potassium chloride (100 mg/Kg) administered intravenously. The procedures were approved by the Ethics Committee in Animal Experimentation (CETEA-UFMG), protocol n˚177/2007. The necropsy was realized immediately after euthanasia and fragments of right ventricle and ear extremity were collected for histopathology and immunohistochemistry. The skin from ear extremity was collected to compare the tissue parasitism of the heart. It is well known that skin is frequently altered and parasited by *Leishmania* sp. in symptomatic dogs (6). Tissue samples were fixed in 10% neutral buffered formalin for 24 hours and then processed for paraffin embedding and stained with hematoxilin and eosin (HE) or further processed for imunodetection of *L. chagasi*. Deparaffinized slides were hydrated and incubated in 4% hydrogen peroxide (30 v/v) in 0.01 M PBS, pH 7.2, followed by incubation with normal goat serum (diluted 1:100). Canine hyperimmune serum from dogs naturally infected with *L. chagasi* (diluted 1:100 in 0.01 M PBS) was used as primary antibody. Slides were incubated for 18-22 h at 4˚C in a humid chamber. After washing in PBS, the slides were incubated with goat anti-mouse and anti-rabbit biotinylated (Link-DAKO, LSAB2 kit, California, USA), washed again in PBS and incubated with streptoavidin-peroxidase complex (Link-DAKO, LSAB2 kit, California, USA) for 20 min at room temperature. The reaction was revealed by 0.024% diaminobenzidine (DAB, Sigma, St. Louis, USA) and 0.16% hydrogen peroxide (40 v/v). Finally the slides were dehydrated, cleared, counter-stained with Harris´s hematoxilin.

The macroscopic findings found in this case are in according to the literature (3). At the necropsy, classical lesions of cachexia, generalized lymphadenopathy, splenomegaly with white and red pulp hyperplasia, splenitis, multifocal nephritis, dermatitis, alopecia, ulcerated lesions, mainly in ear extremity and onychogryphosis were observed. Mild dilated cardiomiopathy and moderate hydropericardium were also present. Microscopically, a multifocal to coalescent and moderate to intense infiltration of lymphocytes, plasma cells and macrophages was detected in approximately one third of the pericardium. Intracytoplasmatic amastigotes within macrophages associated with pericarditis were observed (Fig. 1). The tissue parasitism was confirmed through positive imunomarcation (Fig. 2). A discrete and focal chronic inflammation was present in the myocardium. This animal had also an intense and diffuse chronic dermatitis, predominantly in the upper dermis with many macrophages containing immunolabeled amastigotes, focal ulcer and mild hydropic degeneration of the epidermis in the ear extremity.

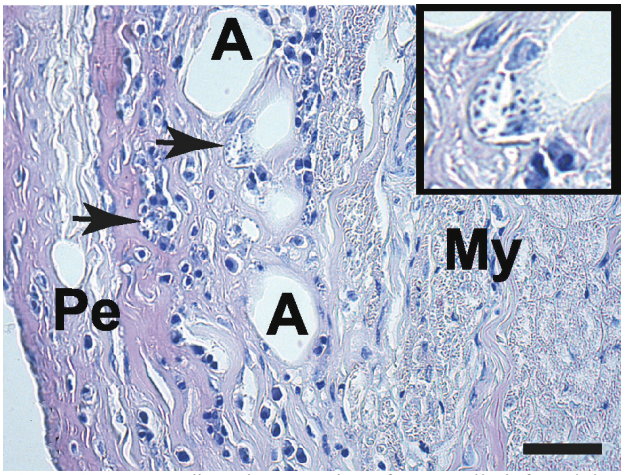

Figure 1 – Myocardium tissue section of a naturally infected dog with *L. (L.) chagasi:* observe a chronic inflammatory infiltration and parasitized macrophages in the pericardium (arrows). (Pe) Pericardium; (My) Myocardium. HE (Bar = 16μm).

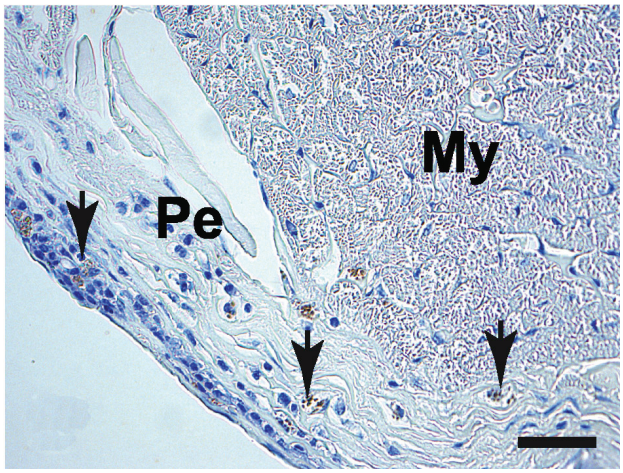

Figure 2 – Myocardium tissue section of a naturally infected dog with *L. (L.) chagasi:* intracytoplasmatic amastigotes within macrophages associate with pericarditis (arrows). (Pe) Pericardium; (My) Myocardium. Streptoavidin-Peroxidase (Bar = 16 μm).

In our study was described an atypical heart parasitism of *Leishmania chagasi* associated with chronic and extensive pericarditis of a naturally infected adult dog in Brazil. These cardiac changes should be considered possible complications in immunocompromised dogs with visceral leishmaniasis.

Acknowledgements

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